

# Density and types of probiotic bacterial filter media with different number of bioballs in silver pompano (*Trachinotus blochii*) culture with recirculation system

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**Abstract.** Cultivation intensification with a recirculation system tends to have a high stocking density and a large amount of feed, so that feed residue, metabolic waste, and oxygen consumption also increase. To speed up the decomposition of feed residue and metabolic waste, filtering is necessary, namely by means of a bioball. This study aimed to analyze the types and numbers of bacteria present in the bioball, to improve water quality and the optimal number of bioballs for bacterial living media. The study used a completely randomized design, with 1 factor, 5 levels of treatment and 3 replications. The treatment levels are: A=without bioballs (control), B=35 bioball filters per container, C=45 bioball filters per container, D=55 bioball filters per container and E=65 bioball filters per container. Silver pompano (*Trachinotus blochii*) fish specimens measuring 10-12.55 cm and a body weight of 24.3-28.9 g, were cultivated for 56 days, at a stocking density of 1 fish for a water volume of 4 L (a total of 20 fish in a volume of 80 L). During the rearing, fish were fed with Megami GR2 pellets with a composition of 46% protein, 10% fat, 2% crude fiber and 10% moisture content, 3 times a day ad libitum. The type and density of bacteria in the bioball was observed, together with the water quality parameters, such as: temperature, pH, dissolved oxygen, total ammonium nitrogen, nitrite and nitrate. The research data were analyzed statistically using ANOVA ( $P < 0.05$ ), Newman Keuls's advanced test and multiple regression models, with the SPSS 17.0 software. The best treatment was found in the use of 55 bioball filters per containers, with a volume of 9.8 L, which gave a bacterial density of  $667.67 \pm 1.53 \times 10^5$  CFU  $\text{mL}^{-1}$ . The number of bioballs affected the bacterial density by 31.1%, the nitrogen absorption in fish by 16.5% and the filter media by 85.2%. The bacteriocidal types found in the filter media are *Acinetobacter* sp., *Shewanella* sp. and *Nitratireductor* sp. A salinity of 15‰ in the culturing media with a filter substrate of 55 bioball filters per container in the recirculation system gave the best bacterial density and water quality: temperature 28.6-29.1°C, pH 7.6-7.8, dissolved oxygen (DO) 6.3-6.7  $\text{mg L}^{-1}$ , total ammonia nitrogen (TAN) 0.06-1.57  $\text{mg L}^{-1}$ , nitrite ( $\text{NO}_2$ ) 0.120-0.861  $\text{mg L}^{-1}$  and nitrate ( $\text{NO}_3$ ) 0.93-3.07  $\text{mg L}^{-1}$ .

**Key Words:** bioball, bacterial density, probiotics, water quality.

**Introduction.** Silver pompano (*Trachinotus blochii*) fish is a marine aquaculture species that is relatively new in Indonesia. However, the demand for this kind of fish continues to increase, especially from the international market. *T. blochii* is a highly adaptable fish species, easy to cultivate and with a considerable economic value. Its consumption price reaches USD 4.25  $\text{kg}^{-1}$  on the local markets and USD 14.144  $\text{kg}^{-1}$  as export commodity (Mo 2017). Since 2015, *T. blochii* has become a leading commodity in marine cultivation fisheries, with a production of 1900 tons in 2015 and the target to increase production with 31.5%  $\text{year}^{-1}$  (Prahadi 2015).

The production target for the *T. blochii* commodity can be achieved through the culture intensification, but the high stocking density results in a higher potential for stressing, due to a narrower space and to an increased competition for oxygen and feed, so that the fish growth will be hampered. High density also accelerates the decline in water quality due to the accumulation of metabolic waste and feed residue, shaving a major effect on growth.

Nitrogen from fish culture waste produces ammonia ( $\text{NH}_3$ ), due to feed residue and fish metabolism, which can result in a buildup of organic matter causing a decrease

in water quality (Putra & Pamukas 2011; Prayogo et al 2012). Recirculation systems are technologies used to maintain water quality in fisheries, in order to remain suitable for aquatic organisms and to support the optimization of water utilization. The recirculation system is able to reduce the level of ammonia concentration, restraining it within the range of 31-43% (Djokosetiyanto et al 2006; Putra & Pamukas 2011).

According to Lekang (2008) and Fadhil et al (2010), the use of a recirculation system has several advantages including: more efficient water use, flexibility in culture locations, more hygiene, relatively small space or land requirements, ease of controlling and maintaining cultured organisms, ease of maintaining water quality, environmentally friendly, pollution prevention and it can be functional permanently, without disturbing the aquatic environment.

Recirculating aquaculture system technology can also be adapted to the aquaculture system and to the filter substrate in order to control the dissolved solids (Fadhil et al 2010). The use of the right type of filter material will determine the success of fish rearing in the recirculation system because it will determine the growth of non-pathogenic bacteria on the material. Currently, biological filter substrates are widely used, being more environmentally friendly.

There are many biological filters that can be used, one of which is the bioball. Bioball is a sphere with a diameter of 4 cm, a specific area of  $\pm 230 \text{ m}^2 \text{ m}^{-3}$  with a width of each hole in a bioball of 0.92 mm, made of PVC material (Said 2002). Bioball functions as a physiological filter which is a growing medium for bacteria that can remove ammonia contained in water (Alfia et al 2013). Nelvia et al (2015) reported that the bioball filtered *Nitrosomonas* sp. bacteria, which function to oxidize ammonia to nitrite, and *Nitrobacter* sp., which function to oxidize nitrite to nitrate.

Several studies related to improving the water quality in aquaculture with recirculation and hydroponic systems have been carried out, such as: Mulyadi & Pamukas (2011) used a recirculation system in nursery and rearing of fish (*Mystus nemurus*); Alfia et al (2013) used a recirculation system with a bioball filter in tilapia (*Oreochromis niloticus*) culture; Nelvia et al (2015) studied the addition of a bioball to the maintenance media filter on the survival and growth of goldfish (*Carassius auratus*) fry. Based on the description above, a series of studies on the number and types of bacteria cultured with different numbers of bioballs and on the improvement of water quality in the culturing and recirculation systems are needed. This research was aimed at analyzing the types and numbers of bacteria present in the bioball, to measure the water quality improvement (temperature, pH, DO, TAN, nitrite and nitrate) and to determine the optimal number of bioballs.

## Material and Method

**Location and time of research.** This research was conducted between January and July 2019, in several laboratories, as follows: the fish rearing was carried out at the Batam Marine Cultivation Fishery Center (BPBL), the water quality analysis was carried out at the Laboratory of Fish and Environmental Health Testing of the Batam Marine Aquaculture Fisheries Agency, the bacteria types identification and densities calculation were performed at the Fish and Environmental Health Examiners Lab of the BPBL, the polymerase chain reaction (PCR) test was performed at PT Genetics Science Indonesia, West Jakarta.

**Materials and tools.** The main ingredients used in this research were 300 seeds of *Trachinotus blochii* (Lacepede) with a size of 10-11 cm, originating from the BPBL, fed with commercial pellets "GR-2" (46% protein, 10% fat, 13% ash content, 2% crude fiber and 10% moisture content). The test feed was obtained from the BPBL. A round bucket with a diameter of 60 cm, a height of 45 cm and a volume of 100 L was used as a research vessel. Bioballs have a diameter of 4 cm, a specific area  $\pm 230 \text{ m}^2 \text{ m}^{-3}$  and a width of each hole in a bioball of 0.92 mm, and are made of PVC, as a filter substrate (Figure 1a).

**Experimental design.** The research method used in this study was an experimental method completely randomized design, with 1 factor, 5 levels of treatment and 3 replications (Steel & Torrie 1993). As a factor in the study was the difference in the number of bioballs. The treatment level determination in this study is based on the study of Nelvia et al (2015) on the goldfish fry (*Carassius auratus*) in closed recirculation system, stating that the use of 50 bioballs gutter<sup>-1</sup> can improve the water quality, providing 100% survival rates and a growth performance up to a weight of 2.605 g and to a length of 1.48 mm. Therefore, the treatment in the present study was established as follows: A=0 (control), B=35, C=45, D=55 and E=65 bioball filters per container.

**Research procedure.** *T. blochii* measuring 10-12.55 cm in length and having a body weight of 24.3-28.9 g were obtained from the BPBL and were adapted for 7 days before being used as test specimens. Fish rearing containers were filled with sea water with a salinity of 15‰, using a closed recirculation system. The rearing container was connected to a 50 x 14 x 14 cm PVC gutter, as a filter container placed at the top of the fish-raising containers. Then the water from the filter gutter flowed back through the PVC pipe with a diameter of 2.5 cm to the rearing tub of *T. blochii*. Water from the fish rearing containers flowed into the filter media (gutters), each gutter containing a number of bioballs corresponding to the tested treatment (Figure 1b) and according to Nelvia et al (2015), by using a water pump with a power of 18 watts. After passing through the filter media (gutter), the water returned to the fish-raising tub through the drain pipe in the filter container. In the rearing medium (KP-SUPER N, with *Nitrosomonas* sp. and *Nitrobacter* sp.), trademark starter bacteria were added at a dose of 2.5 mL 200 L<sup>-1</sup> week<sup>-1</sup> (Hartini et al 2013), to accelerate bacterial growth in the bioball.



Figure 1. (a) Bioball used as a filter substrate, (b) The design of the *Trachinotus blochii* aquaculture recirculation system (original).

The test specimens that have been adapted to the culturing media were then randomly placed into 15 rearing containers. The fish were given commercial feed at satiation, containing: 46% protein content, 10% fat, 13% ash, 2% crude fiber, and 10% moisture, at 07:00, 12:00 and 17:00 WIB.

**Measured response.** The response variables measured in this study were: density and type of bacteria in the filter container were observed at the beginning, in the middle and at the end of the study (SNI.01-2332.3-2006 and SNI.01-2332.3-2006); the temperature, pH and salinity were observed every day; the dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite (NO<sub>2</sub>) and nitrate (NO<sub>3</sub>) were observed every week. Water quality measurement procedures refer to SNI in the Public Works Service (2013).

**Data analysis.** Bacterial density data were analyzed according to the RAL model (Steel & Torrie 1993). To determine the effect of salinity on each measured variable, a diversity analysis was performed using the SPSS 17.0 software. If  $P < 0.05$ , the Newman-Keuls further test was performed to see the differences between treatments. Water quality data were analyzed descriptively using the Microsoft Excel application and displayed in tables and graphs. To see the relationship of the number of bioballs and the density of bacteria with the water quality, a regression analysis was carried out.

## Results and Discussion

**Density of bacteria in the filter container.** The results of bacterial density observations carried out on plate count agar (PCA) media using the total plate count method can be seen in Figure 2.

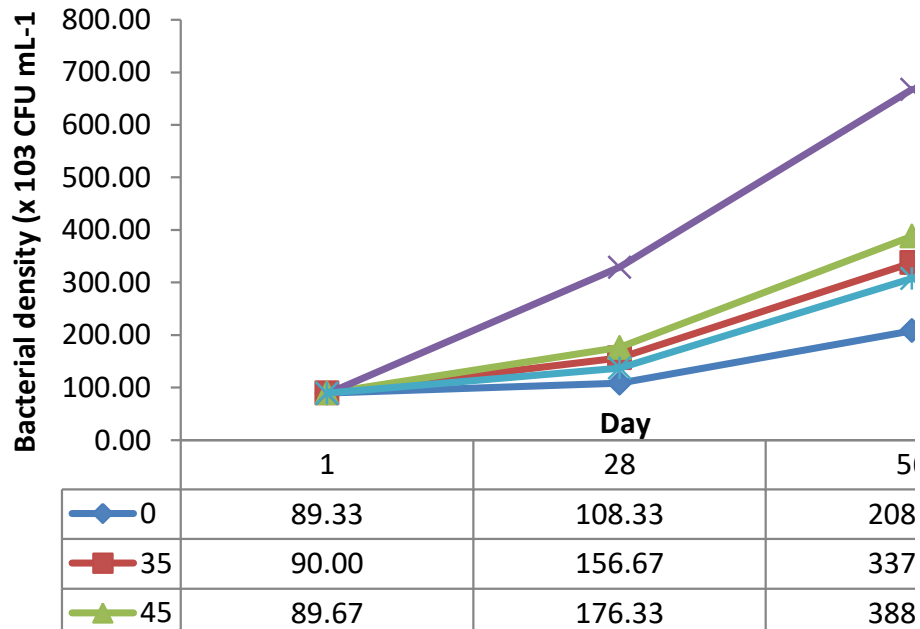


Figure 2. Bacterial density in all treatments during the study.

Figure 2 shows that the bacterial density from day 1 to day 28 in treatments with 0, 35, 45 and 65 bioball filters per container are relatively similar, in contrast to the treatment with 55 bioball filters per container, which caused a significant increase of bacteria, compared to the other treatments. This is due to the fact that the bacteria and *T. blochii* fish are permanently adapting to the media in which they live and to the fact that the nutrients resulting from the remaining feed and fish feces are still not sufficient to support the maximum bacterial growth. After the 28<sup>th</sup> day the number of bacteria in all treatments continued to increase, reaching a maximum peak on the 56<sup>th</sup> day. This is due to the increase in organic matter from fecal matter and fish feed along with the growth of the *T. blochii* specimens, which is a source of bacterial nutrition, thereby increasing the rate of bacterial development. In accordance with the opinion of Sunarto (2003), dissolved organic matter is needed by living bacteria, then Sidharta (2000) explains that organic material contains carbon, nitrate, phosphate, sulfur, ammonia, and several minerals which are nutrients for bacterial growth. Administering KP SUPER N probiotics (routinely added in culture containers) causes continuous bacterial growth due to the availability of organic materials that can be used optimally. The highest bacterial density was found in the treatment of 55 bioball filters per container.

The highest bacterial density was found in the treatment with 55 bioball filters per container, at the end of the study, namely  $667.7 \times 10^5 \text{ CFU mL}^{-1}$ , demonstrating the optimality of a configuration consisting of: 55 bioballs for the given size of the filter (50 x 14 x 14 cm) and for the given volume of the culture media (80 L). These parameters are balanced in such a manner that bacteria receive a sufficient amount of oxygen, through the bioball cavity. According to Nelvia et al (2015), the bioball structure (not too compact) provides an opportunity for oxygen to enter the bioball cavity and to reach bacteria. If the oxygen is sufficient, then the bacteria growth determines an optimal functioning of the bioball.

The lowest bacterial density,  $208.3 \times 10^5 \text{ CFU mL}^{-1}$ , was found in treatment A without using bioballs, starter bacteria (KP SUPER N) and additional nutrients, resulting in a limited media for the growth and development of bacteria. The low density of

bacteria when using the highest number of bioball filters (65 container<sup>-1</sup>) is due a number of bioballs almost exceeding the container capacity. According to Nelvia et al (2015) bioball too densely disposed in the gutter and lacking of oxygen supply can cause extinction of the bacteria in the bioball cavity. Consequently, the performance of the bioball filter decreases, suspending the process of decomposing organic matter by nitrifying bacteria.

The biofilter system removes ammonia where it accumulates and reaches toxic levels if it is not transferred by a nitrification process where the process, first oxidizing ammonia to toxic nitrite, then to nitrate, which is relatively non-toxic. The above process involves *Nitrosomonas* and *Nitrobacter* bacteria. Bacteria grow like a film on the surface of the material (biofilter container) or adhere to organic particles. Biofilter consists of a medium with a large surface for the growth of nitrite bacteria. In the recirculation system, bacteria play a role in converting toxic substances (ammonia) to non-toxic substances (nitrate). The performance of bacteria in the biofilter system is marked by an increase in the average biochemical oxygen demand (BOD) value in the culture medium (Nurhidayat et al 2012). The more bioballs, the more opportunities for bacteria to stick, when a viable oxygen level is still preserved (Nelvia et al 2015).

ANOVA results showed that the number of bioballs had an effect on the density of bacteria on day 28 and 56. The Newman Keuls range study test identifies the bacterial density differences between treatments. The results of the regression analysis between the number of bioballs and bacterial density provide the following regression equation:

$$Y = 227.9 + 3.851X$$

Where:

Y-bacterial density;

X-number of bioballs;

The calculated r was 0.557 and the R<sup>2</sup> was 0.311.

The results of the regression analysis showed that the number of bioballs had a positive correlation with the bacterial density (r=0.557). The increase in the number of bacteria in the filter container was influenced by the number of bioballs by 31.1 and 68.9%, which is thought to be influenced by the content of organic matter (Stepwise regression analysis). Nelvia et al (2015) stated that the bioball is a breeding ground for various bacteria needed in the breakdown of organic matter. According to Sunarto (2003), dissolved organic matter is needed by bacteria to live. Furthermore, according to Sidharta (2000) organic matter contains carbon, nitrate, phosphate, sulfur, ammonia and several minerals which are nutrients for bacterial growth.

**Identification of bacteria in filter containers.** Based on the observations, there were 7 pure bacterial isolates. The identification of bacteria against the isolates found in the filter media was carried out by physical, biochemical and sensitivity tests to see which bacteria had the greatest inhibition zone against pathogenic bacteria.

**Physics test.** The results of observations of color, shape, edge, elevation and gram of the seven isolates found are presented in Table 1.

Table 1

Observation results of bacterial characteristics

No	Isolate	Colony				Cell		Treatment
		Color	Shape	Edge	Elevation	Gram type	Shape	
1	A <sub>1</sub>	Yellow	Circular	Entire	Umbonate	Negative	Stem	P <sub>2</sub> U <sub>1</sub>
2	A <sub>2</sub>	Beige	Circular	Entire	Raised	Negative	Stem	P <sub>0</sub> U <sub>3</sub>
3	A <sub>3</sub>	White	Circular	Entire	Raised	Negative	Stem	P <sub>3</sub> U <sub>2</sub>
4	A <sub>4</sub>	White	Circular	Entire	Raised	Negative	Stem	P <sub>4</sub> U <sub>1</sub>
5	A <sub>5</sub>	Beige	Filamentous	Entire	Filiform	Negative	Stem	P <sub>1</sub> U <sub>1</sub>
6	A <sub>6</sub>	White	Circular	Entire	Raised	Negative	Stem	P <sub>4</sub> U <sub>2</sub>
7	A <sub>7</sub>	White	Circular	Entire	Raised	Negative	Stem	P <sub>3</sub> U <sub>3</sub>

Table 1 shows that all bacterial isolates have gram-negative, entire edges and are rod-shaped. The dominant bacterial colony was white (4 isolates), 2 isolates were beige and 1 isolate was yellow. Colonies were circular or round (6 isolates) and filamentous (1 isolate). The elevation of the dominant bacterial colony was raised (5 isolates), 1 umbonate isolate and 1 filiform isolate.

**Biochemical test.** The results of observations of the Catalase, Oxidase, Motility, oxidative/fermentation (O/F) glucose, Indole and triple sugar iron agar (TSIA) tests are presented in Table 2.

Table 2  
Bacterial biochemical test results

No.	Isolate	Biochemical characteristics						Treatment
		Catalase	Oxidase	Motility	O/F	Indole	TSIA	
1	A <sub>1</sub>	+	+	+	-	-	A/K	P <sub>2</sub> U <sub>1</sub>
2	A <sub>2</sub>	+	+	-	F	-	K/K	P <sub>0</sub> U <sub>3</sub>
3	A <sub>3</sub>	+	-	+	O	-	A/K	P <sub>3</sub> U <sub>2</sub>
4	A <sub>4</sub>	+	+	+	F	-	A/A	P <sub>4</sub> U <sub>1</sub>
5	A <sub>5</sub>	+	+	+	F	+	A/A	P <sub>1</sub> U <sub>1</sub>
6	A <sub>6</sub>	+	+	+	F	-	A/A	P <sub>4</sub> U <sub>2</sub>
7	A <sub>7</sub>	+	+	-	F	-	A/A	P <sub>3</sub> U <sub>3</sub>

O-oxidative; F-fermentative; A/K-alkaline acid; K/K-alkaline; A/A-acid.

Table 2 shows a positive catalase enzyme test in all bacterial isolates, indicated by the gas bubbles formation during their reaction with the H<sub>2</sub>O<sub>2</sub> drops. According to Stoica (2016), catalase is an enzyme contained in the majority of bacteria and it is involved in the decomposition of hydrogen peroxide into water and oxygen. Hydrogen peroxide and oxygen are used for electron transport during fermentation in both aerobic and facultatively anaerobic bacteria.

Oxidase tests A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub> and A<sub>7</sub> are positive, as shown by the color change on the oxidase paper, which indicates activity, while A<sub>3</sub> is negative. Five isolates (A<sub>1</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub>) showed positive results in the motility test, due to the spread of bacterial growth on the Sulfide Indole Motility (SIM) medium, and did not grow on the part of the stick site bacteria. The O/F test results showed no color change in the paraffin-covered media and a color change on the exposed media. Five bacterial isolates (A<sub>2</sub>, A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub>, A<sub>7</sub>) were observed to be fermentative because the tube without paraffin and the tube filled with paraffin changed their color to yellow. According to Fahri (2008), in the O/F test, oxidative organisms occur at a color change in the open media, while fermentative organisms can be indicated by no color change in the closed media.

The indole test shows 6 isolates (A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>6</sub> and A<sub>7</sub>) giving negative results, marked with a yellow color on the surface of the media, which means that the bacteria are unable to break down the amino acid tryptophan, and no red ring after dropping the Kovacs reagent in the sulfur, indole, motility (SIM) media. According to Acharya (2012), the indole test was carried out to determine the ability of bacteria to break down amino acid tryptophan and to form indole compounds. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products, one of which being the indole. Indole production is detected by the Kovacs or Ehrlich's reagent, composed of 4-(dimethylamino)benzaldehyde, which reacts with the indole to produce a red compound. A positive reaction is indicated if there is a red color on the surface of the medium, which indicates that the bacteria are able to break down the amino acid tryptophan. In the TSIA test, 4 isolates (A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub> and A<sub>7</sub>) showed acidic properties (A/A), 2 isolates (A<sub>1</sub> and A<sub>3</sub>) were acid alkaline and 1 isolate (A<sub>2</sub>) was alkaline.

**Sensitivity test.** The sensitivity test to pathogenic bacteria was carried out after observing the physical and biochemical tests of the seven isolates. This test aimed to find probiotic bacteria candidates for polymerase chain reaction (PCR) testing. The results of the sensitivity test on the 7 isolates are presented in Figure 3. Inhibition zones produced



by the seven bacterial isolates were found in different filter containers. Bacteria from isolate A<sub>1</sub> were able to inhibit the growth of *Vibrio* bacteria during the 24-hour incubation period at a temperature of 37<sup>0</sup>C, as indicated by the formation of a clear area around the blank disk, but they were unable to inhibit the growth of *Aeromonas* and *Pseudomonas* bacteria. After an incubation period of 48 hours, the inhibition zone remains clear, which indicates that these bacteria can kill (bacteriocidal) *Vibrio* bacteria. Similarly, for the bacteria from isolate A<sub>2</sub>, a clear zone was formed around the blank disk, indicating an inhibition of *Pseudomonas* and *Vibrio* bacteria during the 48-hour incubation period, meaning that A<sub>2</sub> bacteria are bacteriocidal against *Pseudomonas* and *Vibrio*, but they cannot inhibit the growth of *Aeromonas* bacteria.

Bacteria in A<sub>3</sub> and A<sub>4</sub> isolates were unable to inhibit the growth of *Aeromonas*, *Pseudomonas* and *Vibrio* bacteria, as shown by a no inhibition zone formed on the blank disk. In A<sub>5</sub> and A<sub>7</sub> isolates, an inhibition zone occurred against *Aeromonas*, *Pseudomonas* and *Vibrio* bacteria during the 24-hour incubation period, but during the 48-hour incubation period, the clear area became cloudy, indicating that these bacteria were only able to inhibit (bacteriostatic) the three pathogenic bacteria. In A<sub>6</sub> isolate, a clear zone was also formed during the 48-hour incubation period against *Aeromonas* and *Vibrio* bacteria, but no inhibition zone was formed against *Pseudomonas* bacteria. This indicates that the A<sub>6</sub> bacteria are able to kill (bacteriocidal) *Aeromonas* and *Vibrio* bacteria. This is in line with Azaldin et al (2020), who states that sensitivity of a material to bacteria is characterized by the presence of an inhibition zone around the blank disk. Furthermore, according to Dwyana & Murniati (2020), this inhibition area is formed due to bioactive compounds contained in microbes that produce exoenzymes which break down organic matter and secrete hydrolytic enzymes, proteases, lipases, amylases, and cellulose so that they are able to hydrolyze polysaccharides as a carbon source and electron donors.

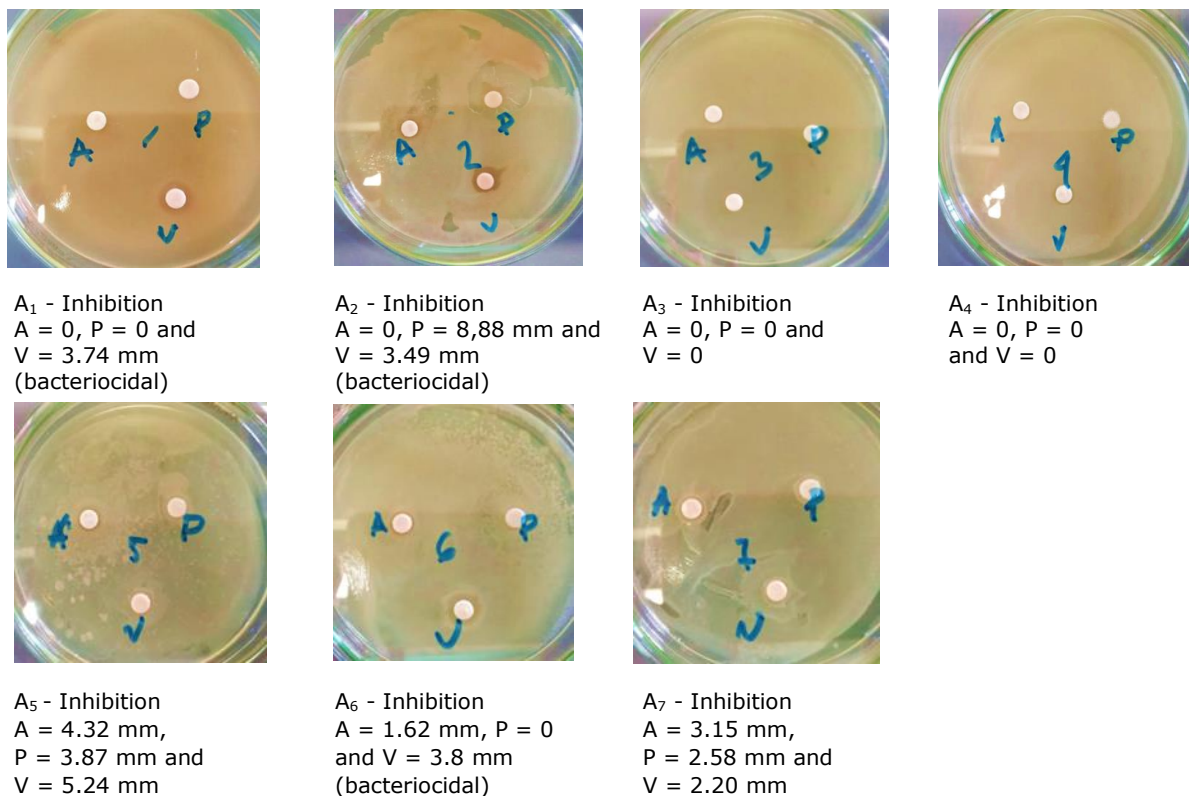


Figure 3. Sensitivity test of bacterial isolates against pathogenic bacteria (A: *Aeromonas*, P: *Pseudomonas*, V: *Vibrio*).

Madigan et al (2012) grouped bacteria based on their selective toxicity. According to them, antimicrobial compounds had 3 kinds of effects on the microbial growth: 1). Bacteriostatic inhibit growth but they do not kill. Bacteriostatic compounds inhibit protein synthesis or bind to ribosomes; 2). Bacteriocides kill cells but not cause cell lysis or cell

breakdown. 3). Bacteriolytic cause cells lysis or cell breakdown. Furthermore, Dwyana & Murniati (2020) stated that the sensitivity test on bacteria indicated that these were bacteriostatic after 24 hours of incubation period (clear zone formed on disc paper), while after 48 hours they were classified as bacteriocidal.

According to Jannata (2014), the responses related to the bacterial growth inhibition can be classified based on the inhibition zone diameter, as follows: weak ( $\leq 5$  mm), medium (5-10 mm), strong (10-20 mm) and very strong ( $\geq 20$  mm). Based on this scale, isolates A<sub>5</sub>, A<sub>6</sub> and A<sub>7</sub> were classified as having a weak response to *Aeromonas*, isolate A<sub>2</sub> had a moderate response and isolates A<sub>5</sub> and A<sub>7</sub> had a weak response to *Pseudomonas* bacteria. Furthermore, A<sub>5</sub> isolate had a moderate response to *Vibrio* bacteria, while isolates A<sub>1</sub>, A<sub>2</sub>, A<sub>6</sub> and A<sub>7</sub> were classified as having a weak response. According to Sugita et al (1996), these bacteria are able to produce antimicrobial compounds, such as: siderophores, bacteriocins, lysozymes, proteases, hydrogen peroxidase, and organic acids, as a mechanism of competition for nutrients and energy, inhibiting the growth of pathogens in the filter container. According to Rengpipat et al (2000), the siderophore is a compound with a low molecular weight ( $<1500$ ) and it is also a specific agent for ferric ions binding, which can dissolve iron precipitates and change them into the form required for microbial growth. An organism's ability to form siderophores causes inhibition in other organisms, especially in aquatic animal pathogens, due to their incapacity to obtain the required ferric ions.

Based on the results of observations on the inhibition zone diameter and inhibition power, the bacterial isolates selected as candidates for probiotic bacteria were: A<sub>1</sub>, A<sub>2</sub> and A<sub>6</sub>, classified as bacteriocidal, then coded MUL\_1, MUL\_2 and MUL\_3. Furthermore, to determine the type of the three bacteria, a DNA analysis was carried out using PCR.

### **Polymerase chain reaction (PCR) test**

**The results of amplification and visualization of DNA fragments of the 16S rRNA gene.** The genomic DNA had been isolated and extracted from isolates MUL\_1, MUL\_2 and MUL\_3, and then the 16S rRNA markers were amplified using PCR technique. PCR analysis of the 3 bacteria isolates used a 27F forward primer: AGAGTTTGATCMTGGCTCAG and reverse primer 1492R: TACGGYTACCTTGTTACGACTT. The analysis results showed positive with the formation of bands with a total molecular weight (in base pairs - bp) of 1,000–1,500 bp. The results of amplified DNA visualization, using 27F and 1,492R primers electrophoresed with UV light, are presented in Figure 4. The amplified fragment has a molecular weight of 1,227-1,372 bp. Sequence analysis performed with Geneious reveals that isolates produce single clearly visible bands with a molecular weight of 1,331 bp (MUL\_1), 1,227 bp (MUL\_2) and 1,372 bp (MUL\_3), according to the primary amplification size with 27F and 1,492R primers, by comparison using 1 Kb Ladder DNA. The size is in accordance with the expected size of the bacterial 16S rRNA genes, which is between 1,300–1,500 bp (Seprianto et al 2017).

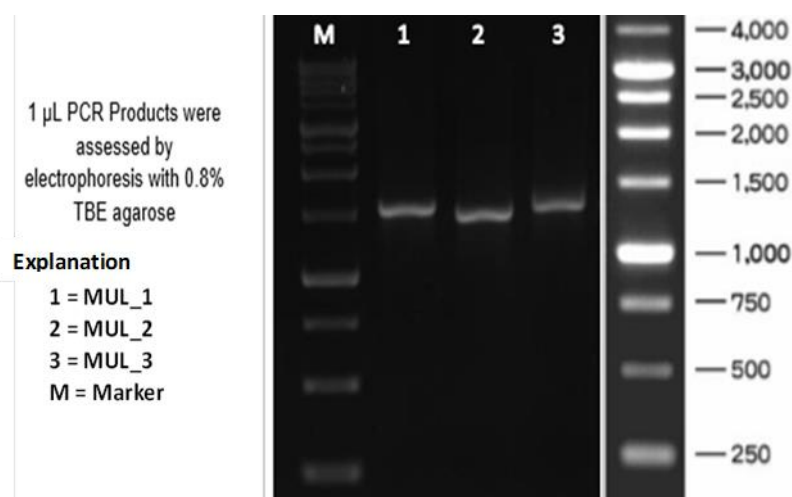


Figure 4. Results of universal DNA-PCR amplification on agarose gel.





Information (NCBI), the species, strains and accession numbers of bacterial isolates identified in this study are presented in Table 3.

Table 3

Results of tracing the 16S rRNA sequencing of bacterial isolates with the BLAST system

<i>Isolate</i>	<i>Species</i>	<i>Strain</i>	<i>Accession number</i>	<i>Query coverage</i>	<i>Homology</i>
MUL_1	<i>Acinetobacter</i> sp.	MUL37	MT229070	100%	100%
MUL_2	<i>Shewanella</i> sp.	MUL31	MT229068	100%	100%
MUL_3	<i>Nitratireductor</i> sp.	MUL35	MT229069	100%	100%

The alignment results of 16S rRNA gene sequences with BLAST (Table 3) showed that the bacterial isolate MUL\_1 was identified as *Acinetobacter* sp. strain MUL37 with 100% homology and 100% query coverage, MUL\_2 isolate was identified as *Shewanella* sp. strain MUL31 with 100% homology and 100% query coverage and bacterial isolate MUL\_3 was identified as *Nitratireductor* sp. strain MUL35 with 100% homology and 100% query coverage. The types of bacteria found are thought to have come from the waters of the Batam Sea which are used as a medium for culturing *T. blochii*, because the starter bacteria added to the media do not contain these types of bacteria.

According to Adithiya et al (2017), the sequence equation between 93-97% can represent the identity of bacteria at the genus level but different at the species level. Furthermore, Hagstrom et al (2000) and Seprianto et al (2017) state that bacteria that have a 16S rRNA sequence equation greater than 97% are the same species, while the equation between 93-97% can represent identity at the genus level but differ at the species level. According to Dancourt et al (2000), if the similarity of the sequences is less than 97%, it is likely either a new species, since there is no data in the database, or the the sequencing results are too short to be relevant, when compared to the database.

**Phylogenetic tree analysis.** The analysis of phylogenetic tree formation was carried out by looking at the similarities that exist in the genetic isolates of MUL\_1, MUL\_2 and MUL\_3 bacteria with a database of the results of alignment of nucleotide sequences in GenBank using the Mega X 10.0.5 WIN 64 application. Phylogenetic trees analysis used the UPGMA (Unweighted Pair Group Method with Arithmetic Average), a clustering method based on the pairwise distance arithmetic means. Phylogenetic analysis used 17 sequences with 3 sample sequences and 14 comparison sequences, the alignment process used 1000 bootstraps and the Kimura 2 model parameters. Branches that connect the dots (nodes) are taxonomic units, such as species or genes and tree roots are the ancestors for all organisms. The dendrogram is presented in Figure 5.

Figure 5 shows that the bacteria MUL\_1, MUL\_2 and MUL\_3 are in 1 cluster and that they are separated from the type of control bacteria, which means that MUL\_1 and MUL\_2 have close relationships, tending even to be of the same species or to belong to the same subspecies, while MUL\_3 bacteria are from the same genus. Based on the the branching point's location, it could be considered that the three isolates were closely related to *Nitratireductor* and *Shewanella*.

According to the calculation results of the genetic distance (using Mega X 10.0.5 WIN 64), it was found that the distances between species varied, ranging from 0.00 to 1.6659. The smallest genetic distance, of 0.2005, was found between bacteria MUL\_1 and MUL\_2, while between MUL\_1 and MUL\_3 it was of 0.2081. The genetic distances found were the following: 1.2531 between bacteria MUL\_1 and *Acinetobacter venetianus* (GenBank: NR.042049.1), 1.2955 between MUL\_2 and *Shewanella algae* (GenBank: NR117771.1) and 1.2468 between MUL\_3 and *Nitratireductor aquimarinus* (GenBank: 117929.1). According to Tallei et al (2016), the smaller the genetic distance between two organisms, the closer their kinship.

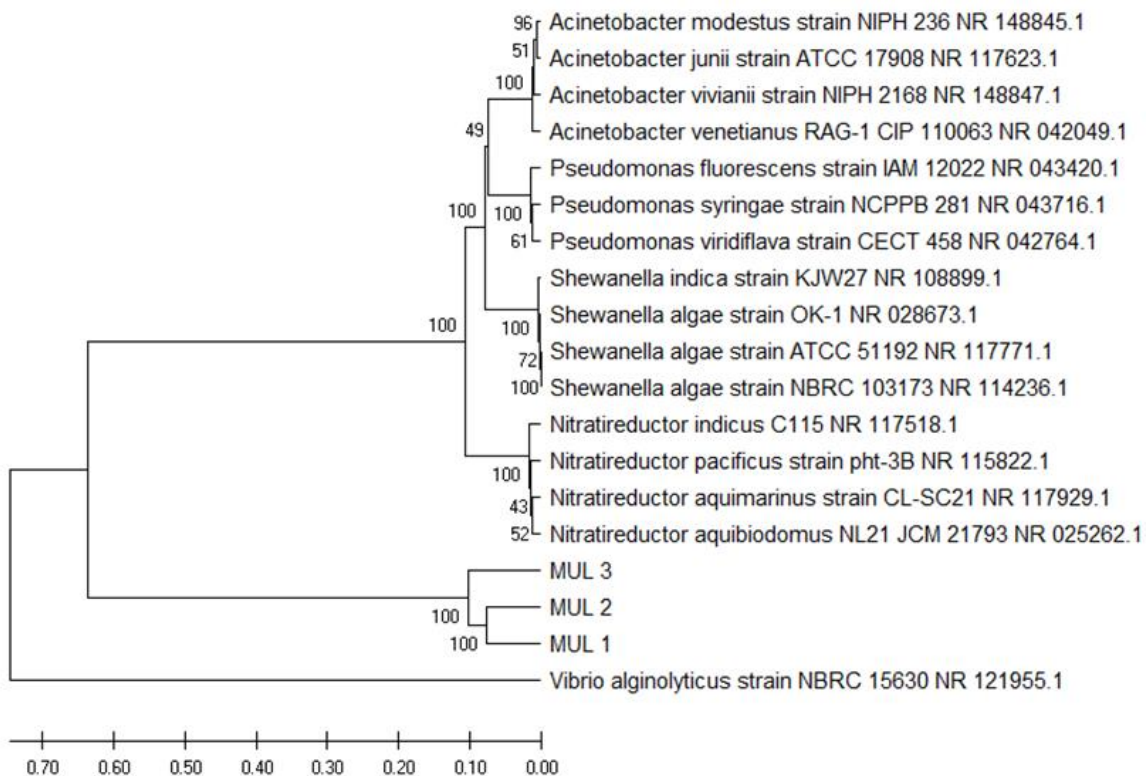


Figure 5. Phylogenetic tree dendrogram of isolates MUL\_1, MUL\_2 and MUL\_3 with 16 comparative species.

Bauvet & Grimont (1986) stated that *Acinetobacter* (Isolate MUL\_1) belongs to the kingdom *Acinetobacter*, phylum Proteobacteria, class Gammaproteobacteria, order Pseudomonadales, family Moraxellaceae, genus *Acinetobacter*. In general, these bacteria are rod-shaped, gram-negative, non-motile, catalase positive and oxidase negative, live aerobically, and are opportunistic. Beleneva & Maslennikova (2004) and Soslau et al (2011) reported that the *Acinetobacter* bacteria were found to be associated with several invertebrates such as mollusks, sea cucumbers, sea urchins, starfish and crustaceans.

*Shewanella* bacteria (Isolate MUL\_2) belong to the Bacteria kingdom, the Proteobacteria phylum, the Gamma Proteobacteria class, the Alteromonadales order, the family Shewanellaceae and the genus *Shewanella*. The special characteristics of Shewanellae are their ability to capture electrons in oxygen deficient conditions and their ability to survive in various habitats (Gralnick & Newman 2007). The genus *Shewanella* is a gram-negative rod-shaped proteobacteria, 2-3  $\mu\text{m}$  long and 0.4-0.7  $\mu\text{m}$  in diameter. These bacteria are facultative anaerobic bacteria that are commonly found in marine sediments and can swim with the help of a single polar flagellum (Venkateswaran et al 1999). *Shewanella* is a genus of metal reducing bacteria found in marine environments, freshwater, lakes, land or terrestrial, rivers, Arctic and Antarctic oceans, corroded oil pipes and aquifer environments contaminated with uranium. These bacteria are widely used for bioremediation or environmental cleaning of pollutants such as chlorinated compounds, radionuclides and other environmental pollutants (Venkateswaran et al 1999).

According to Labbe et al (2004), *Nitratireductor* (Isolate MUL\_3) belongs to the kingdom Bacteria, phylum Proteobacteria, class Alpha Proteobacteria, order Rhizobiales, family Phyllobacteriaceae and genus *Nitratireductor*. *Nitratireductor* bacteria isolated from sea water in the Indian Ocean and marine sediments in the Pacific Ocean have the ability to reduce ammonia, are rod-shaped and form white bacterial colonies. The optimum temperature for their growth is 25-35°C and pH 5-12.

**Water quality.** The results of observations on temperature, pH, dissolved oxygen (DO), TAN, nitrite, (NO<sub>2</sub>) and nitrate (NO<sub>3</sub>) in all treatments during the study are presented in Table 4.

Table 4

Average temperature, pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite (NO<sub>2</sub>) and nitrate (NO<sub>3</sub>) in all treatments during the study

Parameters	Number of bioball of each filter container				
	0	35	45	55	65
Temp. (°C)	28.6-29.1	28.4-29.1	28.7-29.1	28.6-29.1	28.6-29.1
pH	7.5-7.7	7.6-7.7	7.6-7.7	7.6-7.8	7.6-7.7
DO (mg L <sup>-1</sup> )	6.2-6.5	6.2-6.6	6.2-6.6	6.3-6.7	6.2-6.6
Salinity (g L <sup>-1</sup> )	15	15	15	15	15
TAN (mg L <sup>-1</sup> )	0.61-1.58	0.28-1.59	0.18-1.56	0.06-1.57	0.12-1.56
Nitrite (mg L <sup>-1</sup> )	0.624-0.953	0.209-0.863	0.170-0.875	0.120-0.861	0.129-0.874
Nitrate (mg L <sup>-1</sup> )	0.820-1.12	0.98-1.40	0.96-2.15	0.93-3.07	0.98-1.42

Table 4 shows that all water parameters, namely: temperature, pH, dissolved oxygen (DO), ammonia (NH<sub>3</sub>) and nitrite (NO<sub>2</sub>) are relatively similar in all treatments, except for the nitrate content. The highest nitrate content was found in the treatment with 55 bioball filters per container. In general, temperature, pH, DO, TAN, nitrite and nitrate are in a good range for the growth of *T. blochii*.

SNI 7901.4 (2013) and Ashari et al (2014) state that the optimal temperature for the growth of *T. blochii* ranges from 28 to 32°C, and the pH ranges from 6.8 to 8.4. According to Ezraneti et al (2019), *T. blochii* fish can live optimally at a salinity of 14-19 ppt. SNI 7901.4 (2013) requires a dissolved oxygen level of at least 5 mg L<sup>-1</sup> for the rearing of *T. blochii* seeds. Royan et al (2019) reported that the lethal concentration (LC50) of TAN ranges from 1.10 to 22.8 mg L<sup>-1</sup> for invertebrates and from 0.56 to 2.37 mg L<sup>-1</sup> for fish, within 24-96 hours after exposure. The nitrite level for the maintenance of *T. blochii* seeds is of maximum 1 mg L<sup>-1</sup> (SNI 7901.4. 2013).

The highest nitrate concentration was found in the treatment with 55 bioball filters containers<sup>-1</sup> for a volume of 9.8 L, optimal for nitrification bacterial culture media. The higher the number of bacteria, the faster the nitrification process, resulting in an increase in the concentration of nitrate in the media. According to Lampert & Sommer (2007), bacteria have proteolytic enzymes that can break down organic nitrogen into nitrates, which are nutrients needed by phytoplankton and other aquatic microflora. Furthermore, Nelvia et al (2015) obtained the almost the same optimal number of bioballs for the growth of goldfish, namely 50 bioball filters per container of 9.8 L. Nitrate levels obtained during the study were still in accordance with the quality standards referred to in the Government Regulation No. 82 of 2001, namely <20 mg L<sup>-1</sup>. Effendi (2014) considers a nitrate content of 0-1 mg L<sup>-1</sup> in the oligotrophic (low) category and a content of 1-5 mg L<sup>-1</sup> in the mesotrophic (moderate) category. According to Ulqodry et al (2010), normal nitrate content in marine waters generally ranges from 0.01-50 mg L<sup>-1</sup>. Furthermore, Hartoko (2010) reports that algae, especially phytoplankton, can grow optimally at a nitrate content of 0.009-3.5 mg L<sup>-1</sup>.

**Relationship between number of bioballs and water quality.** The results of the regression analysis between the number of bioballs with temperature, pH, DO, TAN, NO<sub>2</sub> and NO<sub>3</sub> are presented in Table 5.

Table 5 shows that the number of bioballs has the greatest effect on the TAN reduction (R<sup>2</sup>=0.931 or 93.1%), with a very strong negative linear correlation (r=0.965), meaning that the ammonia concentration decreases by adding bioballs in the filter container. This is because in the filter media there are several types of nitrifying bacteria derived from the introduced starter bacteria, namely *Nitrosomonas* sp. and *Nitrobacter* sp., in addition to the bacteria found in the bioball, namely *Acinetobacter* sp., *Shewanella algae* and *Nitratireductor aquimarinus*. The presence of these bacteria can accelerate the

oxidation process of TAN, which is toxic to fish, into non-toxic nitrate through the nitrification process.

Table 5

Linear regression analysis between the number of bioballs with temperature, pH, DO, TAN, NO<sub>2</sub> and NO<sub>3</sub>

Parameter	Linear regression equation	Correlation coefficient (r)	Coefficient of determination (R <sup>2</sup> )
Temperature	Y=289.72-0.038 X	-0.767	0.589
pH	Y=759.51+0.067 X	0.444	0.197
Dissolved oxygen (DO)	Y=634.56+0.196 X	0.650	0.423
TAN	Y=58.29-0.83 X	-0.965	0.931
Nitrite (NO <sub>2</sub> )	Y=587.55-8.345 X	-0.949	0.900
Nitrate (NO <sub>3</sub> )	Y= 94.73+1.75 X	0.664	0.441

The value of r=0 indicates that there is no correlation between the two variables (number of bioballs and water quality), r<0.25 indicates a very weak correlation, r>0.25 and <0.5 shows a sufficient correlation, r> 0.5 and <0.75 suggest a strong correlation, r>0.75 and <0.99 demonstrate a very strong correlation and r=1 signifies a perfect correlation; R<sup>2</sup>≥0.5 (50%) measures the ability to explain the dependent variable (water tanquality) by the independent variable (number of bioballs) (Sarwono 2012).

**Relationship between bacterial density and water quality.** The results of regression analysis between bacterial density and temperature, pH, DO, TAN, NO<sub>2</sub> and NO<sub>3</sub> are presented in Table 6.

Table 6

Linear regression analysis between bacterial density with temperature, pH, DO, TAN, NO<sub>2</sub> and NO<sub>3</sub>

Parameter	Linear regression equation	Correlation coefficient (r)	Coefficient of determination (R <sup>2</sup> )
Temperature	Y=289.50-0.003 X	-0.226	0.475
pH	Y=756.37+0.015 X	0.695	0.484
Dissolved oxygen (DO)	Y=289.50+0.036 X	0.816	0.667
TAN	Y=59.578-0.090 X	-0.725	0.526
Nitrite (NO <sub>2</sub> )	Y=557.73-0.796 X	-0.625	0.391
Nitrate (NO <sub>3</sub> )	Y=30.304+0.352 X	0.923	0.852

Table 6 shows that bacterial density has the greatest influence on the increase in nitrate concentration in the *T. Blochii* rearing medium (R<sup>2</sup>=0.852 or 85.2%). This is due to the presence of *Nitrosomonas* bacteria, *Nitrobacter* which are included as a starter in the filter container, and to the heterotrophic bacteria in the bioball, which accelerate the ammonia to turn into nitrate, so that the nitrate concentration increases with the bacterial density.

**Conclusions.** The number of bioballs had an effect (P<0.05) on the bacterial density. The best treatment was found in the use of 55 bioball filters per container, in a volume of 9.8 L, which gave a bacterial density of 667.67±1.53 x 10<sup>5</sup> CFU mL<sup>-1</sup>. The number of bioballs had an influence on the bacterial density by (R<sup>2</sup>=31.1%). The bacteriocidal types found in the filter media are *Acinetobacter*, *Shewanella* and *Nitratireductor*. The number of bioballs strongly affected the decrease in TAN (R<sup>2</sup>=93.1%), while the bacterial density strongly influenced the increase in nitrates (R<sup>2</sup>=85.2%).

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## References

- Acharya T., 2012 Oxidase test: principle procedure and oxidase positive organisms. <https://microbeonline.com/oxidase-test-principle-procedure-and-oxidase-positive-organisms/>
- Adithiya D. S., Feliatra F., Afrizal T., 2017 [Using of bacteria heterotrophic as an anti-bacterial againts pathogenic bacteria isolated from sea water in Dumai city, Riau Province]. *Jurnal Online Mahasiswa Perikanan dan Kelautan* 4(2):1-17. [In Indonesian].
- Alfia A. R., Arini E., Elfitasari T., 2013 [The effect of different densities on livelihoods and growth of tilapia (*Oreochromis niloticus*) in the recirculation system with bioball filters]. *Journal of Aquaculture Management and Technology* 2(3):86-93. [In Indonesian].
- Ashari S. A., Rusliadi, Putra I., 2014 [Growth and livelihoods of silver pompano fish (*Trachinotus blochii*, Lacepede) with different stocking densities reared in floating net cages]. <https://media.neliti.com/media/publications/199307-none.pdf>. [In Indonesian].
- Azaldin M., Syawal H., Lukistyowati I., 2020 [Sensitivity of pineapple peel (*Ananas comosus*) extract against *Edwardsiella tarda* bacteria]. *Jurnal Ruaya* 8(1):53-59. [In Indonesian].
- Bauvet P. J. M., Grimont P. A. D., 1986 Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. Nov., *Acinetobacter johnonii* sp. nov., *Acinetobacter junii* sp. nov., and emended description of *Acinetobacter calcoaceticus* and *Acinetobacter iwoffii*. *International Journal of Systematic Bacteriology* 36(2):228-240.
- Beleneva I. A., Maslennikova E. F., 2004 Spread of bacteria of the genus *Acinetobacter* in the hydrobios of the bay of peter the great (the Sea of Japan). *Journal of Microbiology, Epidemiology and Immunobiology* 3:88-90.
- Dancourt M., Bollet C., Carlioz A., Martelin R., Gayral J. P., Raoult D., 2000 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *Journal Of Clinical Microbiology* 38:3623-3630.
- Djosetiyanto D., Sunarma A., Widanarni, 2006 [Changes of ammonia, nitrite and nitrate at recirculation system of red tilapia (*Oreochromis* sp.) rearing]. *Jurnal Akuakultur Indonesia* 5(1):13-20. [In Indonesian].
- Dwyana Z., Murniati, 2020 [Sensitivity test of probiotic bacteria against *Vibrio harveyi* in vitro causes of vibriosis]. *Journal of Natural and Environmental Sciences* 11(2):15-23. [In Indonesian].
- Effendi H., 2014 [Study of water quality for management of aquatic environmental resources]. Kanasius, Yogyakarta, Indonesia, 258 p. [In Indonesian].
- Ezraneti R., Adhar S., Alura A. M., 2019 [Effect of salinity on physiological conditions in silver pompano fish seeds (*Trachinotus blochii*)]. *Acta Aquatica: Aquatic Sciences Journal* 6(2):52-57. [In Indonesian].
- Fadhil R., Endan J., Taip F. S., Ja'afar M. S. H., 2010 [Recirculating aquaculture system technology to increase inland fishery production in Aceh: an overview]. Aceh Development International Conference, Auditorium Hall, Faculty of Engineering, Universiti Putra Malaysia, pp. 826–833. [In Indonesian].
- Fahri M., 2008 [Pathogenic bacteria of *Vibrio alginolyticus* in aquaculture]. MSc Thesis, Mahasiswa Program Pascasarjana, Brawijaya University, Malang, Indonesia, 66 p. [In Indonesian].
- Gralnick J. A., Newman D. K., 2007 Extracellular respiration. *Molecular Microbiology* 65:1–11.
- Hagström A., Pinhassi J., Zweiefel U. L., 2000 Biogeographical diversity among marine bacterioplankton. *Aquatic Microbial Technology* 21:231-244.
- Hartini S., Sasanti A. D., Taqwa F. H., 2013 water quality, survival rate and growth of snakehead (*Channa striata*) maintained in media with addition of probiotics. *Jurnal Akuakultur Rawa Indonesia* 1(2):192-202.
- Hartoko A., 2010 [Oceanography and marine fisheries resources in Indonesia]. UNIDP



- PRESS, Semarang, 466 p. [In Indonesian].
- Jannata R. H., Gunadi A., Ermawati T., 2014 [Antibacterial activity of manalagi apple peel (*Malussylvestris* mill.) extract on the growth of *Streptococcus mutans*]. Jurnal Pustaka Kesehatan 2(1):1-9. [In Indonesian].
- Labbe N., Parent S., Villemur R., 2004 Nitratireductor aquibiodomus gen. nov., sp. nov., a new alpha-proteobacterium isolated from the marine denitrification system of the montreal biodome (Canada). International Journal of Systematic and Evolutionary Microbiology 54:269-273.
- Lampert W., Sommer U., 2007 Limnoecology: the ecology of lakes and streams. 2<sup>nd</sup> edition. Oxford University Press, New York, 324 p.
- Lekang O. I., 2008 Aquaculture engineering. Blackwell Publishing Ltd., Oxford, UK, 340 p.
- Madigan M. T., Martinko J. M., Stahl D. A., Clark D. P., 2012 Brock biology of microorganisms. Pearson, San Fransisco, 8 p.
- Mo Y., 2017 [Looking at the astrology potential of silver pompano]. www.isw.co.id. [In Indonesian].
- Mulyadi, Pamukas N. A., 2011 [Optimalisation of water for nursery and rearing of catfish (*Mystus nemurus* C.V)]. Proceeding 2<sup>nd</sup> International Seminar of Fisheries and Marine Science, Faculty of Fisheries and Marine Science, University of Riau, 107-118. [In Indonesian].
- Nelvia L., Elfrida, Basri Y., 2015 [Addition of bioball to the filter media on growth and survival of goldfish seeds (*Carassius auratus*)]. Jurnal Manajemen Universitas Bung Hatta 7(1): 1-12. [In Indonesian].
- Nurhidayat, Nirmala K., Djokosetyanto D., 2012 [Performance effectiveness of biofilter media in the recirculation system on water quality for growth and survival of red rainbow fish (*Glossolepis incises* Weber)]. Jurnal Riset Akuakultur 7(2):279-292. [In Indonesian].
- Prahadi Y. Y., 2015 [Production of silver pompano is targeted to grow 31.5%]. Swa.co.id./swa/trends/management. [In Indonesian].
- Prayogo, Rahardja B. S., Manan A., 2012 [Exploration of indigenized bacteria in a closed recirculation system of African catfish (*Clarias* sp.) hatchery]. Jurnal Ilmiah Perikanan dan Kelautan 4(2):193-198. [In Indonesian].
- Putra I., Pamukas N. A., 2011 [Maintenance of selais (*Ompok* sp.) fish with recirculation, aquaponic system]. Jurnal Perikanan dan Kelautan 16(1):125-131. [In Indonesian].
- Rengpipat S., Rukpratanporn S., Piyatiratitivorakul S., Menasaveta P., 2000 Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (Bacillus S11). Aquaculture 191:271-288.
- Royan M. R., Solim M. H., Santanumurti M. B., 2019 Ammonia-eliminating potential of *Gracilaria* sp. and zeolite: a preliminary study of the efficient ammonia eliminator in aquatic environment. IOP Conference Series: Earth and Environmental Science. 236(1):012002.
- Said N. I., 2002 [Biofilter application for small industrial wastewater management]. Cetakan 1. BPPT, Jakarta, Indonesia, 18 p. [In Indonesian].
- Sarwono J., 2012 Quantitative approach thesis research method using SPSS procedures (1<sup>st</sup> edition). Jakarta: PT Elex Media Komputindo, 308 p. [In Indonesian].
- Seprianto, Feliatra, Nugroho T. T., 2017 [Isolation and identification of probiotic bacteria from the intestine of tiger shrimp (*Penaeus monodon*) based on 16S rDNA gene sequences]. Jurnal Ilmiah Biologi Biogenesis 5(2):83-92.
- Sidharta B. R., 2000 [Characteristics of marine bacteria; introduction to marine microbiology]. Universitas Atmajaya, Yogyakarta, Indonesia, 13 p. [In Indonesian].
- Soslau G., Russell J. A., Spotila J. R., Mathew A. J., Bagsiyao P., 2011 *Acinetobacter* sp. HM746599 isolated from leatherback turtle blood. FEMS Microbiology Letters 322:166-171.
- Steel R. G. D., Torrie J. H., 1996 Principles and procedures of statistics: A biometrical approach subsequent edition. McGraw-Hill College, 672 p.
- Stoica C., 2016 Biochemical test & method for bacterial identification; KOH-Test. <http://www.tgw1916.net/Tests/nitrates.html>
- Sugita H., Kawasaki J., Kumazawa J., Deguchi Y., 1996 Production of amylase by the

- intestinal bacteria of japanese coastal animals. Letter in Applied Microbiology 23: 174-178.
- Sunarto, 2003 [The role of decomposition in the production process in marine ecosystems]. <http://www.rudyct.com/PPS702-ipb/07134/sunarto.pdf> [In Indonesian].
- Tallei T. E., Rembet R. E., Pelealu J. J., Kolondam B. J., 2016 Sequence variation and phylogenetic analysis of *Sansevieria trifasciata* (Asparagaceae). Bioscience Research 13(1):01-07.
- Ulqodry T. Z., Yulisman, Syahdan M., Santoso, 2010 [Characteristics and distribution of nitrates, phosphates, and dissolved oxygen in the waters of Karimunjawa, Central Java]. Jurnal Penelitian Sains 13(1):35-41. [In Indonesian].
- Venkateswaran K., Moser D. P., Dollhopf M. E., Lies D. P., Saffarini D. A., Gregor B. J. M., Ringelberg D. B., White D. C., Nishijima M., Sano H., Burghardt J., Stackebrand E., Nealson K. H., 1999 Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. International Journal of Systematic Bacteriology 49:705-724.
- \*\*\* Standard National Indonesia, SNI, 2013 [Silver pompano (*Trachinotus blochii*, Lacepede) - part 4: seed production]. National standardization agency for Indonesia. Jakarta, Indonesia, 8 p. [In Indonesian].

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